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Product-ion Assisted Library Search of the Electron Ionization Mass Spectrum of a Mixture

Jian-Ting Chen, Chiun-Gung Juo, Chia-Fu Yeh and Guor-Rong Her*

Department of Chemistry, National Taiwan University, Taipei, Taiwan

It has been found that fragment-ion spectra derived from molecular ions do not, in general, achieve good scores when matched against standard library mass spectra. Although the same *m/z* values may be observed, the relative intensities are frequently too different from those in the EI mass spectral libraries. A strategy has been developed for compound identification in simple mixtures, in which fragment-ion spectra of molecular ions are adjusted to match the relative intensities of the same *m/z* values in the EI mass spectrum of the mixture. It is shown that this simple adjustment permits more reliable identification of compounds in a mixture by library matching than does use of the unadjusted fragment-ion spectra.

The major use of gas chromatography/mass spectrometry (GC/MS) lies in the identification and quantitation of 'unknowns' in complex mixtures.[1–4] One important reason for the rapid growth of GC/MS is the development of automated methods, such as library searching, for identification of compounds eluting from the GC. These developments[5–8] greatly reduce the time and expertise required in the interpretation of mass spectra. Nowadays, almost all commercial GC/MS systems are equipped with programs for automatic matching.

An important factor in library searching is the size and quality of the reference library. In acquiring electron impact (EI) mass spectra, it is relatively easy to control instrumental conditions so that the spectra recorded from different instruments are sufficiently similar. Therefore, a high-quality EI reference library can be constructed, and 'unknown' spectra can be matched successfully against this reference library. Many different algorithms have been proposed for matching unknown spectra against EI reference libraries.[8–14] The probability based matching (PBM) system, due to its availability on commercial mass spectrometer data systems, has gained widespread use. Though most often used for mass spectra of pure compounds, PBM with the spectrum-stripping technique has been used for the analysis of mixtures with up to three components.[15]

Tandem mass spectrometry (MS/MS) represents another powerful technique for mixture analysis. In the context of mixture analysis, techniques without prior chromatographic separation are especially useful for compounds which are not amenable to GC/MS analysis. Unlike EI mass spectra, however, library searching has not been widely applied to product-ion mass spectra[16–18] because of the lack of a high-quality reference library containing a wide range of compounds. This is due, at least in part, to the fact that product-ion mass spectra are known to be much more sensitive to instrumental conditions, therefore making the construction of a high-quality product-ion reference library a difficult task.

A new approach to analysis of the EI mass spectrum of a mixture has been studied. In this approach, the EI-like mass spectrum of each component in a mixture was created from the product ion spectrum of the molecular ion, using the ion intensities extracted from the mixture's EI mass spectrum. These 'hybrid' spectra could be matched against a standard EI reference library.

EXPERIMENTAL

Chlorobenzilate, alachlor, methomyl and atrazine (Fig. 1) were kindly provided by Hon-ping Lee of Taiwan Agricultural Chemicals and Toxic Substances Research Institute. These pesticides were mixed and prepared as a 50 ppm solution in dichloromethane. Two μL of sample solution was used for each of EI, desorption chemical ionization (DCI) and product-ion analysis.

EI, DCI and product-ion mass spectra (linked-scan at constant B/E) were recorded using a Jeol SX-102A (JEOL, Tokyo, Japan) double focusing mass spectrometer of reversed geometry. Helium was used as the collision gas; the pressure of helium was adjusted to reduce the intensity of ion beam to 30% of its usual value. The mass scale in the linked-scan spectra was calibrated using a mixture of alkali halides.[19]

The PBM algorithm, as provided in the HP MS Chemstation (Hewlett Packard, Palo Alto, CA, USA), was used as the library search system. The NIST/EPA/NIH reference library, which contained 62 235 compounds and 74 828 EI mass spectra, was used.

RESULTS AND DISCUSSION

Despite the fact that many ions present in a standard EI reference spectrum are also observed in the product-ion mass spectrum of the molecular radical cation, product-ion mass spectra are not often successfully searched against standard EI reference library. This is due, in part, to the fact that the relative abundances are often quite different in the two different spectra.

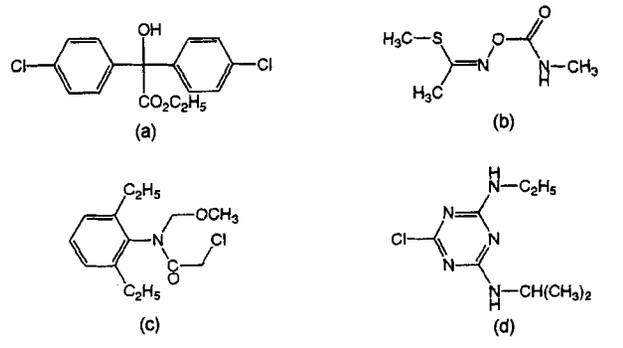

Figure 1. Structures of the four pesticides studied in this work: (a) chlorobenzilate (M.W.=324), (b) methomyl (M.W.=162), (c) alachlor (M.W.=269), (d) atrazine (M.W.=215).

* Author for correspondence.

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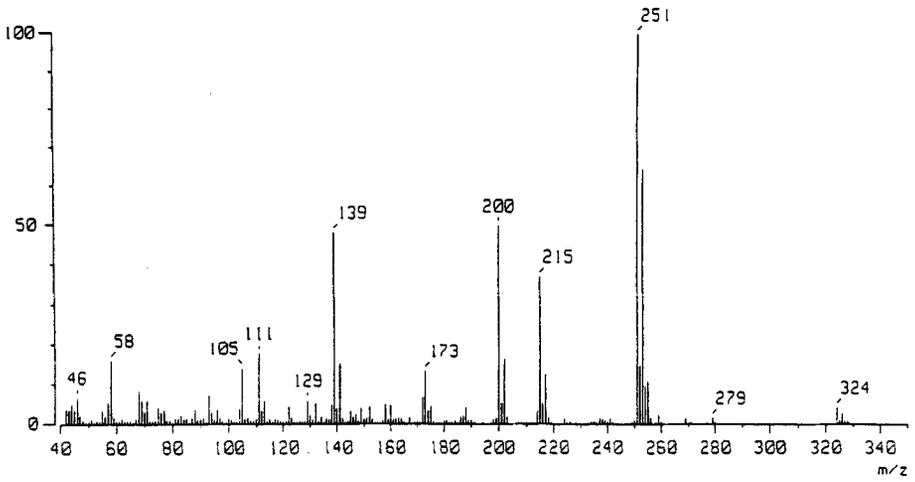

Figure 2. Electron impact mass spectrum of the mixture.

A strategy was developed to circumvent this problem. The procedures may be described as follows:

1. Obtain the EI mass spectrum of the mixture.
2. Obtain the ammonia DCI spectrum of the mixture, to identify the number and molecular weights of the compounds present in the mixture.
3. Obtain the product-ion spectra of the $[M+H]^+$ and/or $[M+NH_4]^+$ ions observed in 2.
4. From the information obtained in 2 and 3, identify in the mixture EI spectrum (1) which ions belong to which compound in the mixture, i.e. group the EI ions together by compound.
5. Obtain product-ion spectra of EI ions categorized in 4.
6. Thus, come up with final lists of *m/z* values of ions in 1 which can be classified together as arising from the same compound.
7. Construct mass spectra for each compound thus identified by its EI ions (1), in which the *m/z* values come from 4–6 but the intensities are those of the same ions as observed in 1.
8. Match these 'EI-like' fragment-ion spectra (in 7) against the available library using the standard library routine (PBM or other).

Because the *m/z* values and relative intensities were extracted from the EI mass spectrum of the mixture, they should have the 'correct' abundances and so have a better chance for a successful match against the standard EI reference library.

Several mixtures have been tested with this approach. A mixture of four pesticides is presented to illustrate the procedures. After acquiring the EI mass spectrum of the mixture (Fig. 2), a DCI experiment using ammonia as reagent gas was used to investigate the number of components in the mixture (Fig. 3). Ammonia DCI gives a very simple CI mass spectrum consisting of $[M+H]^+$ and/or $[M+NH_4]^+$ ions, with very little fragmentation. As shown in Fig. 3, $[M+H]^+$ and $[M+NH_4]^+$ pairs of ions were clear distinguishable for several components (compound A, *m/z* 359/342; B, 287/270; C, 233/216; D, 180/163).

Collision-induced dissociation (CID) experiments were performed on each of the major ions in the DCI mass spectrum (Fig. 3). The first function of these CID experiments is to confirm or to correct the assignment made by DCI. Ions belonging to the same compound can be identified because the product-ion spectra of these ions (protonated molecule, ammonia adduct, and fragment ions) should have many fragment ions in common. In the present case, the CID experiments confirmed the assignments made by DCI, and the ion at *m/z* 308 (Fig. 3) was shown to be a fragment ion of compound A.

The second function of the CID experiments, on the ions produced by ammonia DCI, is to identify the ions in the EI spectrum of the mixture which need to be selected for

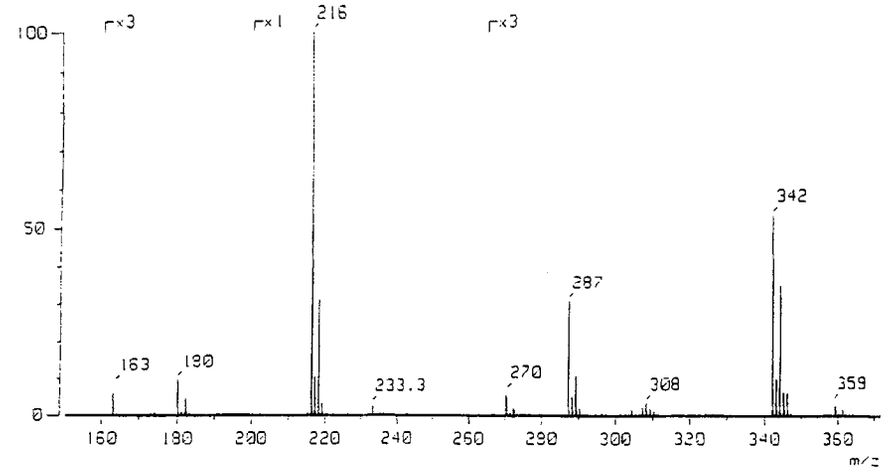

Figure 3. Ammonia DCI mass spectrum of the mixture.

further CID experiments. From these CID mass spectra, it was found that m/z 324, 251 (Fig. 2) were derived from compound A, m/z 269, 188 from compound B, m/z 215, 173 from compound C and m/z 105 from compound D. These ions in the EI mass spectrum of the mixture were selected for further CID experiments. The m/z values of the fragment ions observed in CID experiments were then used to identify those ions in the mixture EI mass spectrum which should be used to construct EI-like fragment-ion spectra for each component in the mixture. These reconstructed spectra were finally matched against the NIST/EPA/NIH reference library using the PBM algorithm. As shown in Fig. 4, the reconstructed and standard library spectra are similar. The results of the search are shown in Table 1. Except for compound D (methomyl), the probability factors for the other three compounds are all over 80

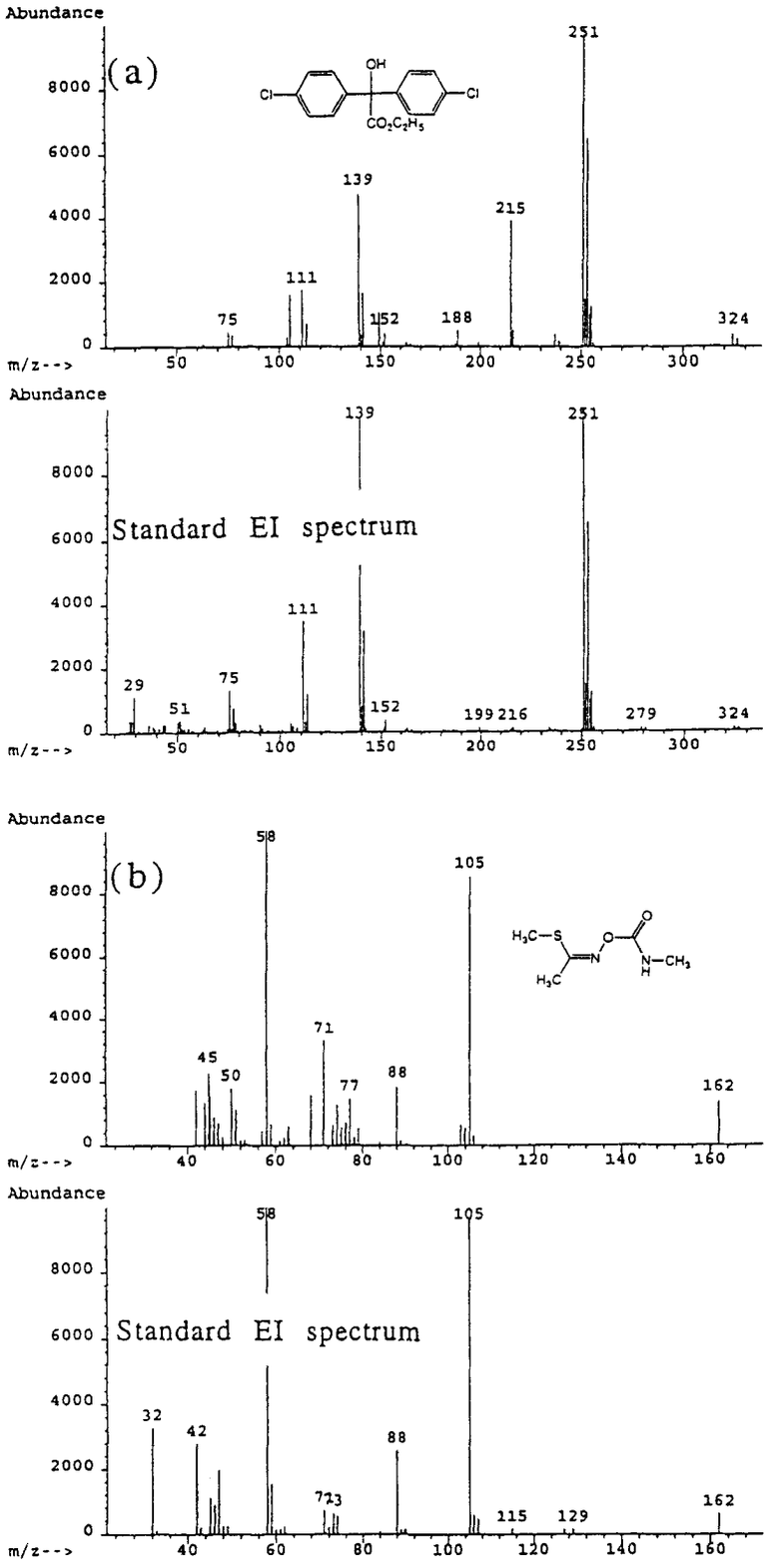

Figure 4. The reconstructed and standard EI mass spectra of (a) compound A (chlorobenzilate); (b) compound D (methomyl). The m/z 215 ion in (a) was an artifact peak, which was co-extracted from compound C.

Table 1. The results of (PBM) library search of reconstructed EI mass spectra.

Name	M.W.	Prob.
Reconstructed EI spectrum (A)		
Chlorobenzilate	324	93
Benzenemethanol, 4-chloro, alpha,-(4-chl	310	64
Chlorobenzilate	324	64
Reconstructed EI spectrum (B)		
Alachlor	269	86
Alachlor	269	47
Alachlor	269	40
Reconstructed EI spectrum (C)		
Atrazine	215	91
Atrazine	215	91
1H-Imidazol-2-amine, 4,5-dihydro-N-(5,6,	215	25
Reconstructed EI spectrum (D)		
Methomyl	162	42
Ephedrine	165	28
Thiirane, (methoxymethyl)-	104	25

(100 is the perfect score). Methomyl appeared to be a difficult case, and the probability was only 62 even for an EI mass spectrum obtained from a pure standard. The information on molecular weight obtained by the DCI experiment was not used for the search. If it is presearched with molecular weight, compounds with incorrect molecular weights (Table 1) will not be listed as potential candidates.

The CID technique used in this work was linked scanning at constant B/E. This technique has good product-ion resolution but poor resolution in the selection of the precursor ion. The poor resolution in precursor-ion selection turned out to be an advantage in that the ^{13}C isotope peak was selected along with the all-^{12}C peak of the precursor ion for CID. The inclusion of ^{13}C isotope peaks in EI mass spectra appeared to be important for the quality of the library search. The disadvantage of the poor resolution in precursor-ion selection is the occurrence of interference (artifact) peaks[20] when analyzing precursor ions with very similar m/z values.

CONCLUSION

An approach to analyzing the EI mass spectrum of a mixture by library searching has been proposed. Preliminary results showed that the EI-like fragment-ion spectra could be successfully matched against an EI reference library. This method may provide an alternative to analysis of mixtures in which all or some of the components are unsuitable for GC/MS analysis but do have their standard EI spectra in the library. This approach could also provide a useful aid for membrane-inlet mass spectrometry (MIMS), a technique of great potential in on-line monitoring without prior chromatographic separation.[21–23] With current instrumentation, this approach is still rather tedious and time consuming. Improvements in automation, both in acquisition of different types of spectra and in establishing EI-like fragment-ion spectra, are needed to make this approach a practical method. Moreover, areas such as (1) components of isobaric mass, (2) fragment ions originating from two or more components in a mixture, (3) mixtures with more than four components, require further study to explore the full potential as well as the limitations of this approach.

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